REVIEWS ARTICLE

Is this a blast? An illustrated practical review on peripheral blood smear examination in the paediatric patient

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Abstract

The morphologic findings on a peripheral blood smear can provide important clues that help establish a diagnosis or guide the workup of many clinical disorders. Finding a blast – whether clinically expected or not – is one of the most impactful of such findings. Pathologists, clinical haematologists, technologists, and trainees in the medical field often feel the need to refer to an illustrated reference when encountering suspected blasts and blast-mimics. This article provides a practical concise resource that demonstrates the morphological features of the various types of blasts and illustrates the cytologic characteristics that help distinguish them from their benign mimickers in the paediatric population.

INTRODUCTION

Arriving at an optimal medical outcome starts with taking a meaningful clinical history followed by performing a thorough physical examination. A common and crucial milestone that follows along this route can often be a careful review of a patient’s complete blood count (CBC). The examination of a well-prepared peripheral blood smear (PBS), whenever possible, can add valuable information to the CBC findings, especially if a haematologic disorder is in the differential diagnosis.

At most major medical centres, morphologic examination of a PBS is performed by a trained technologist and/or a pathologist. The luxury of delegating PBS examination to the pathology service is not universally available to clinicians or consistently possible in a timely fashion. The clinician’s need for self-reliance may arise when advanced pathology services are not available, as in small clinical practices at underserved sites, or even at major hospitals outside regular work hours. In these situations, a level of microscopic familiarity and morphologic skills are needed to enable clinicians to accurately make timely decisions on next steps for the patient. Without such skills, significant delays and/or unnecessary testing, as well as increased cost of care may be incurred.

During a PBS review, one of the most important components may be determining whether a certain cell population or even a single cell is a neoplastic blast or not. While the morphologic skills and experience of the examiner play a major role here, the availability of pertinent educational resources can also be very helpful in guiding the decision process. The purpose of this article is to provide a condensed summary of basic morphologic features of blasts and differentiate them from commonly encountered mimics in the paediatric haematology practice. The text and illustrations are tailored for optimal utilisation by practising clinicians, technologists, and trainees in clinical paediatric haematology and haematopathology.

General morphologic features of a blast

Generally, blasts are larger than their normal counterpart, show higher nuclear to cytoplasmic ratio, variably irregular nuclear contour, smooth or granular chromatin, variably conspicuous single or multiple nucleoli and pale to deep blue cytoplasm. Large size is a feature typical of blasts. The extent of size difference – compared to the normal counterpart – may be widely variable among cases. Myeloblasts, are typically 15-20 µm, while lymphoblasts are generally smaller, and maybe only slightly
larger than a mature lymphocyte. Comparison with a known benchmark cell in the microscopic field can be helpful, and if no other nucleated cells are available for comparison, one can use erythrocytes as a size comparison.

High nuclear-to-cytoplasmic (N/C) ratio is another feature of blasts, which may be quite useful in separating blasts from certain, but not all, atypical lymphocytes. Irregular nuclear contour is also a common feature of blasts. This feature may not be fully appreciated unless the examiner adjusts the fine focus up and down (under high power magnification) to visualise the nuclear membranes’ complex irregularities when present. The fine nuclear chromatin is perhaps the most helpful feature of identifying blasts. This feature gives the blastic nuclei a bland, smooth, and homogeneous appearance, which is a feature not typically seen in normal peripheral blood elements. This especially contrasts normal lymphocytes, which typically possess densely clumped chromatin (exceptions apply: atypical lymphocytes may also show fine nuclear chromatin). Nucleoli are also features seen in blasts, though they are not seen in every blast, and sometimes may be difficult to distinguish. Additionally, they may be found in atypical lymphocytes, immunoblasts, and prolymphocytes.1

The cytoplasm in blasts tends to be pale to deep blue, though their high N/C ratios mean it may be scant and difficult to distinguish. This is different from the greyish-blue of monocytes and the pinker cytoplasm of the maturing granulocytic lineage. If present, azurophilic cytoplasmic granules or Auer rods can help further differentiate myeloblasts from lymphoblasts. Interestingly, there have been rare cases of ALL with cytoplasmic granules reported in the literature.2

Figure 1 shows a mature lymphocyte and a blast, highlighting some of the above morphologic differences. It is important to note that the more of these features are encountered in a cell, the easier it is to recognise it as a blast. However, none of the above features alone is specific or pathognomonic. For example:

1. Large cellular size alone is not very specific, as many mimics (including monocytes and atypical lymphocytes) can be over 20 µm.1
2. Mature lymphocytes have a high N/C ratio, and must be distinguished from blasts.
3. Infant lymphocytes commonly show nuclear membrane irregularities.
4. Atypical lymphocytes may also show fine nuclear chromatin.

It is also important to remember that a well-prepared and stained smear is important for the proper assessment of cells and evaluation of these general characteristics. Typical peripheral blood smears are stained using variants of the Romanowsky stain, which include the Wright, Wright-Giemsa, May-Grünwald Giemsa, and Giemsa stains. Features may vary slightly between these stains – for example, nucleoli appear accentuated on the Wright and Wright-Giemsa stains.3 It is important to be accustomed to the stain which one uses at their institution.

![Figure 1: A lymphoblast (LB) and a mature lymphocyte (L). Note the larger size of blast.](image-url)
BLAST: AN ILLUSTRATED REVIEW ON PERIPHERAL BLOOD SMEAR

Blast mimics encountered in paediatric patients

More commonly in children, blast mimics include atypical reactive lymphocytes and the so-called “infant lymphocytes” (a.k.a. “baby lymphocytes”). Figure 2 compares a normal lymphocyte (Fig. 2A), an atypical reactive lymphocyte (Fig. 2B), and an “infant lymphocyte” (Fig. 2C) with the more common paediatric age lymphoblasts (Fig. 2D). Table 1 lists a comparative summary of the morphologic features of blasts and their mimics.

TABLE 1: Comparative summary of the major morphologic features of blasts and their mimics

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Cell Size</th>
<th>Nuclear/Cytoplasmic Ratio</th>
<th>Nuclear Contour</th>
<th>Chromatin</th>
<th>Cytoplasmic Granules/Auer rods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocyte</td>
<td>≥RBC</td>
<td>High</td>
<td>Smooth</td>
<td>Clumped</td>
<td>No</td>
</tr>
<tr>
<td>Lymphoblast</td>
<td>L1: &gt; RBC</td>
<td>High</td>
<td>Irregular</td>
<td>Smooth</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>L2: &gt;&gt;RBC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>L3: &gt;&gt;RBC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>“Infant Lymphocyte”</td>
<td>≥RBC</td>
<td>High</td>
<td>Irregular</td>
<td>Clumped</td>
<td>No</td>
</tr>
<tr>
<td>Reactive Atypical Lymphocyte</td>
<td>&gt;&gt;RBC</td>
<td>Low</td>
<td>Smooth</td>
<td>Smooth</td>
<td>No</td>
</tr>
<tr>
<td>Myeloblast</td>
<td>&gt;&gt;&gt;RBC</td>
<td>High</td>
<td>Irregular</td>
<td>Granular or Smooth</td>
<td>Possible</td>
</tr>
</tbody>
</table>
Atypical lymphocytes/reactive lymphocytosis on peripheral smears are frequent findings in the differential diagnosis of acute leukaemia in the paediatric population. The paediatric population has a higher baseline lymphocyte count and is more likely to develop brisk reactive lymphocytosis following stimuli, ranging from subclinical insults, to common minor or systemic viral infections. In early childhood, lymphocytes are the predominant leukocyte. As such, lymphocytosis in a child is relatively less worrisome for malignancy than in an adult, where chronic lymphocytic leukaemia/small lymphocytic lymphoma (CLL/SLL) is common.

Morphologically, atypical lymphocytes can be of large size and show worrisome features that mimic blasts. They are most commonly encountered following viral infections, especially EBV, but also with mumps, varicella, syphilis, mycoplasma pneumonia, acute hepatitis, and autoimmune diseases to name a few of the many causes. They may have varying appearances (Fig. 3A-D). The most common variants show large size with extensive blue cytoplasm, nuclear enlargement, and less dense chromatin than normal lymphocytes, an especially vexing feature (Fig. 3A-B). Confusion with acute leukaemia may lead to misdiagnosis; however, features that may be useful in separating the two are a tendency for atypical lymphocytes to have dark blue cytoplasmic accentuation around the cell border, and a propensity for their cytoplasm to project pseudopods or mold to adjacent erythrocytes. Less commonly encountered are the plasmacytoid lymphocytes (Fig. 3C). Atypical lymphocytes with cloverleaf nuclei are rare but strongly correlate with EBV infection (Fig. 3D).

Reactive lymphocytosis with smaller (rather than larger) sized lymphocytes may be seen in certain conditions such as pertussis associated lymphocytosis (Fig. 4). The lymphocytes seen with pertussis infection are small, show nuclear cleaving and convolution, and have scant cytoplasm. This finding is a side effect of the viral toxin causing hyper-maturation of the lymphocyte.

A morphologic variant of the benign lymphocyte, exclusive to the paediatric

![FIG. 3: Examples of reactive atypical lymphocytes. The most common variants show large size with extensive blue cytoplasm, nuclear enlargement, and less dense chromatin than normal lymphocytes (A-B). Atypical lymphocytes have a tendency to have dark blue cytoplasmic accentuation around the cell border, and a propensity for their cytoplasm to project pseudopods or mold to adjacent erythrocytes (B). Less commonly encountered are the plasmacytoid lymphocytes (C). Atypical lymphocytes with cloverleaf nuclei are rare but strongly correlate with EBV infection (D).]
population, are “infant lymphocytes” (Fig. 2C). These are also known as “baby lymphocytes” or “baby lymphs”. They are typically found in children less than 3 months of age and, despite their atypical morphology, are completely benign. They show high N/C ratio and irregular nuclear contours. The “infant lymphocytes” retention of chromatin clumping and normal size may best hint towards their benign nature, in addition to clinical findings and recognition of patient age. Atypical “infant” monocytes can – less commonly – be seen as well in the early months of life (Fig. 5).

Types and subtypes of blasts
In a paediatric setting, acute myeloid leukaemia (AML) and acute lymphoblastic leukaemia (ALL) are the two main malignancies where one will encounter blasts. While blasts can show widely variable morphologies within these two categories, all blasts within them generally share certain morphologic features.

Cytomorphology based knowledge and morphology-based diagnostic skills arguably reached their peak late in the 20th century. At that time, the prevailing morphology-based diagnostic scheme for leukaemia was the so-called French-American-British (FAB)
Classification. With that in mind, a flashback to some of the terms used in the FAB classification can perhaps be helpful.

In the old FAB classification, lymphoblasts were classically divided as L1, L2, and L3 based on morphologic appearance. L1 lymphoblasts (Fig. 6B) are generally on the smaller end of the spectrum (10-14 µm), and contain minimal cytoplasm. These are the blasts that could be most likely confused with mature lymphocytes; however, even the minutest blast should be larger than a mature lymphocyte (Fig. 6A). L2 lymphoblasts (Fig. 6C) have more cytoplasm, though they still have a relatively high N/C ratio. These lymphoblasts tend to have quite irregular nuclear contours and prominent nucleoli. In contrast, L1 lymphoblasts may lack significant nuclear contour irregularity, which is likely attributable to such scarce cytoplasm. Lastly, L3 lymphoblasts (Fig. 6D), or the Burkitt-like lymphoblasts, are intermediate in size, larger than L1 but possibly smaller than L2, and similarly, have an N/C ratio falling between these two. The nuclear contours tend to be less irregular than L2. The most striking feature of these cells is the deeply basophilic cytoplasm, which tends to have significant vacuolisation.

Myeloblasts also have variable appearances which can be divided based on the FAB categorisation. AML M0 (AML with minimal differentiation) myeloblasts usually appear very similar to lymphoblasts and typically cannot be definitively differentiated with morphology alone (Fig. 7A). AML M1 (AML without maturation) myeloblasts tend to have more cytoplasm (resulting in a lower N/C ratio) and may have (≤20) azurophilic granules and possibly Auer rods (Fig. 7B). AML M2 (AML with maturation) myeloblasts have a more mature appearance, with slightly more condensed cytoplasm, and increased (≥20) azurophilic granules and possibly Auer rods (Fig. 7C-D).

An important concept to be aware of is that of “blast equivalents.” These are cell types that are not blasts but are included in the blast count when calculating whether there are ≥20% blasts in the peripheral blood or bone marrow for the diagnosis of acute leukaemia. The major blast equivalents are the promyelocyte in acute promyelocytic leukaemia (APL) and the promonocyte in acute myelomonocytic leukaemia and acute monocytic/monoblastic leukaemia. It is important to note that these only counts towards the blast count for these specific

FIG. 6: The three main morphologic variants of lymphoblasts. Lymphoblasts with L1 morphology are generally on the smaller end of the spectrum (A - compares a lymphoblast, LB, to a normal lymphocyte, L), and contain minimal cytoplasm (B). L2 lymphoblasts (C - arrows) have more cytoplasm, irregular nuclear contours and prominent nucleoli. L3 lymphoblasts (D - arrow heads), or the Burkitt lymphoblasts, are intermediate in size and N/C ratio and have deeply basophilic cytoplasm and frequent nuclear and/or cytoplasmic round vacuoles.
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FIG. 7: AML M0 (AML with minimal differentiation) myeloblasts may appear very similar to lymphoblasts and typically cannot be definitively differentiated with morphology alone (A). AML M1 (AML without maturation) myeloblasts tend to have more cytoplasm (resulting in a lower N/C ratio) and may have azurophilic granules and possibly Auer rods (B). AML M2 (AML with maturation) myeloblasts have a more mature appearance, with slightly more condensed cytoplasm, and are more likely to have azurophilic granules and Auer rods (C-D - arrow head and arrow respectively).

Neoplastic promyelocytes, as seen in APL (AML M3), as well as nonneoplastic promyelocytes are typically larger than myeloblasts (18-25 µm), and have a slightly lower N/C ratio. Auer rods can be seen in most cases, and are typically larger than those in other types of AML. The typical hypergranular variants of APL have variable nuclear morphologies, that are often bilobed or kidney bean-shaped. The typical hypergranular variants have abundant pink-to-purple granules which may obscure the nuclear-cytoplasmic border. Nuclear bi-lobation can be very prominent, giving the nucleus a bifid appearance sometimes referred to as “buttock cells.” Although APL is now translocation-defined, and no longer requires >20% blasts/promyelocytes to make the diagnosis, rare cases exist with typical APL morphology and immunophenotype, that may not have RARα translocation abnormality.

Monoblasts are found in acute myelomonocytic leukaemia (M4) and acute monocytic/monoblastic leukaemia (M5) (Fig. 10C-D). These are usually large and their nuclei can be round to oval. They may have a relatively lower N/C ratio, ranging from 3:1 to 1:1. Their cytoplasm often has a more greyish-blue hue, as opposed to the deeper blue hue commonly seen in other blasts, and may contain fine azurophilic granules and rare vacuoles. These features point towards their monocytic lineage.

Compared to monoblasts, promonocytes are similar in size, but often have a slightly lower N/C ratio, consistent with their more advanced stage of development. The cytoplasm is less basophilic with relatively increased cytoplasmic azurophilic granules and vacuoles compared to monoblasts. Their nuclei are more irregularly shaped and convoluted with delicate folding of the nuclear membranes and a slightly more reticulated chromatin pattern.

Erythroblasts, as seen in erythroid leukaemia (M6), usually have round nuclei, similar to the other cell types of its lineage. Additionally, the chromatin is notable for its distinct pattern, showing more well-defined dark areas and paler pale areas than seen in lymphocytes. These blasts have deeply blue/azurophilic cytoplasm and frequent cytoplasmic vacuoles. Deep blue cytoplasm with vacuoles
FIG. 8: Acute Promyelocytic leukaemia. Auer rods can be seen in most cases of APL, and are typically slender and more numerous than those seen in other types of AML (A). The typical hypergranular variants have abundant pink-to-purple granules which may obscure the nuclear-cytoplasmic border (B). Some variants (AML M3) may have bilobed nuclei (C-D) with scant-to-no visible granules.

FIG. 9: Acute myelomonocytic leukaemia (M4): The peripheral blood may show variable numbers of myeloblasts (arrows) and monoblasts (arrow heads). Confirming the diagnosis requires demonstrating that each blast component represents 20% or more in the marrow.
FIG. 10: Acute myelomonocytic leukaemia (M4) showing myeloblasts (arrows) and monoblasts (A-B - arrow heads). Abnormal eosinophils with basophilic and eosinophilic granules are often seen (B - arrow). Acute monoblastic leukaemia: Monoblasts are usually large and their nuclei can be round to oval. They may have a relatively lower N/C ratio, ranging from 3:1 to 1:1. Their cytoplasm often has a more grayish-blue hue, and may contain fine azurophilic granules and rare vacuoles (C-D).

FIG. 11: Pure erythroid leukaemia. Blasts have deeply blue/azurophilic cytoplasm and frequent cytoplasmic vacuoles (A). Erythroblasts may show unusual cohesiveness (B) or look like pro-erythroblasts with more primitive nuclei (C-D).
are also seen in L3 lymphoblasts – nuclear chromatin differences can help in separating these two entities. Some cases may have pro-erythroblasts showing more primitive nuclei (more traditionally blast-like with fine chromatin) (Fig. 11C-D).

Megakaryoblasts are seen in acute megakaryoblastic leukaemia (M7), classically found in trisomy 21-related leukaemia. Some may have a slightly lower N/C ratio. The most helpful morphologic feature is likely the presence of cytoplasmic, amoeboid, bleb-like projections reminiscent to the budding formation of platelets in normal megakaryocytes1,9 (Fig. 12A-B), discrete giant platelets showing budding themselves (Fig. 12C), and abnormal micro-megakaryocytes with nuclear-cytoplasmic asynchrony, including a dark, central granulomere and a pale, peripheral hyalomere (Fig. 12D).

Tips on efficient examination of peripheral blood smears
A well-prepared PBS should have a generous “reading area.” A reading area is best defined as an area of the slide with evenly spread red blood cells, with neither overlapping nor sizable cell-free gaps. This is where cellular morphology is most optimal for interpretation. The reading area is most easily found by starting at the “feathered edge” of the blood smear (which typically can be identified as the parabolic shape on the slide) and moving away from this until the reading area becomes apparent. When blasts are scant, reviewing the whole slide is obviously necessary, but paying special attention to slide edges is usually most rewarding (for reasons likely related to the laws of physics governing the relationship between cell size and travel distance upon making a smear).

Blasts surrogates
In contrast to the adult population, where nucleated red blood cells (nRBCs) should not be seen in the healthy population, a decreasing number of nRBCs continue to circulate in the peripheral blood of healthy term babies until around day 5 of life.11 Circulating nRBCs may persist longer (weeks or months) in certain clinical contexts, such as prematurity, major congenital infections (TORCH), and conditions associated with certain anaemias or chronic hypoxia.

Beyond early infancy, the presence of nRBCs is abnormal and requires seeking an explanation, including a diligent search to exclude leukemic blasts. The presence of nRBCs in a previously

![FIG. 12: Acute megakaryocytic leukaemia. Primitive blasts showing cytoplasmic budding and/or discrete giant platelets (B-C), and/or abnormal micro-megakaryocytes with nuclear-cytoplasmic asynchrony including a dark, central granulomere and a pale, peripheral hyalomere (D - arrow).](image-url)
healthy child suggests difficulty producing enough erythrocytes to meet the physiological need. This may be due to increased loss of erythrocytes or defective production secondary to any number of causes, including marrow replacement by malignancy. Sills and Hadley found that out of 100 children with circulating nRBCs, 49 had underlying processes associated with hypoxia, and 8 children had malignancies. Interestingly, three of these malignancies (two neuroblastomas and a malignant histiocytosis) were diagnosed while working up the unexplained circulating nRBCs. None of the 400 control patients had circulating nRBCs.

It is important to note that in the proper clinical context, the presence of abnormal promyelocytes and promonocytes is equivalent to blasts in peripheral blood. Haematogones (primitive cells often encountered in the bone marrow of children that can be morphologically misconstrued as blasts) do not circulate in the peripheral blood and hence should not be confused with blasts on PBS.

**Neoplastic vs. regenerative blasts and the importance of clinical context**

The clinical context is essential for recognizing non-neoplastic blasts, as they may be morphologically indistinguishable from neoplastic blasts (Fig. 13A-D). Auer rods are an exception, as they are only found within the context of neoplasia. There are numerous causes of left-shift that may produce blasts in the peripheral blood, including G-CSF administration and toxic marrow injury. Early progenitor cells can be seen in the peripheral blood in cases of erythroleukemoid reaction or during marrow regeneration as seen following chemotherapy. Additionally, the company the blasts keep matter: if there is a spectrum of maturation, this may favour a regenerative proliferation, whereas a disproportionate blast population with no significant number of intermediary cells favours a neoplastic process. A spectrum of maturation is a classic finding in the bone marrow and peripheral blood following chemotherapy, as the haematopoietic cells are in the process of reconstituting the cellularity. This is true for myeloid as well as monocytic cell lines (Fig. 14). Of course, exceptions to this exist, such as in the case of juvenile myelomonocytic leukaemia, where a spectrum of neoplastic hematopoietic cells is seen.

**FIG. 13:** Regenerative blasts (A-D - arrows) may be morphologically indistinguishable from neoplastic blasts. The presence of a spectrum of left-shifted cells and the clinical context can be helpful in recognising non-neoplastic blasts.
Changes in the early days of life
In very premature infants, neutrophils are very scarce in the peripheral blood. Neutrophil count gradually increases from the 20th to 30th weeks of gestation. Samples from these individuals should not be misinterpreted as neutropenia, especially in fetal blood sampling. With low granulocytes in the context of the previously mentioned atypical “infant lymphocytes,” the smear findings could be misconstrued as a leukaemic process without proper context. Recognising the scenario is important for avoiding misdiagnosis. In contrast, neutrophils are often sharply increased in term infants for a brief window following birth. In these individuals, the granulopoietic population shows marked left-shift and circulating blasts may be seen. Rare micro-megakaryocytes may be seen in peripheral blood, which can be misinterpreted as blasts. These transient changes are thought to be due to mobilisation of the available granulocytic population from the marrow due to stress of labour.

FINAL REMARKS
It is important to consider the information presented here as a basic guide, and not a set of iron-clad rules. One should always keep an open mind and try to interpret findings within the clinical context. Some morphologic findings may be impossible to interpret without further testing, such as flow cytometry or cytogenetics.

In situations where the examiner is unable to make definitive conclusions, a note describing the findings and likely possibilities along with a recommendation for follow up testing will be helpful. Time and follow up over time might be needed to allow the process to declare itself, and maybe the best and most cost-efficient ally in determining the nature of rare cells when there is no pressing clinical urgency.

REFERENCES